Original Article

Coalition of E-cadherin and vascular endothelial growth factor expression in predicting malignant transformation in common oral potentially malignant disorders

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Abstract

Background: Reduced E-cadherin expression and increased VEGF expression is known to be involved in tissue growth and transformation of Oral Potentially Malignant Disorders (OPMDs) and has been correlated with their differing histological grades in numerous studies.

Aim: To evaluate Immunohistochemical (IHC) expression of both E-cadherin and VEGF in predicting the malignant transformation potential of common OPMDs.

Materials And Methods: Ten cases each of Normal Oral mucosa (NOM), Mild Oral Epithelial Dysplasia (OED), Moderate OED, Severe OED, Oral Submucous Fibrosis, (OSMF) and Oral Squamous Cell Carcinoma (OSCC) were stained and evaluated for the expression of Ecadherin and VEGF. Quick score (QS) for expression intensity in all epithelial layers was calculated for both markers and results statistically analysed using Kruskal –Wallis ANOVA and Mann-Whitney "U" test.

Results: E-cadherin expression was continuous and membranous in all the layers of NOM and reduced with progressing grades of OED to OSCC. In OSMF, expression was intermediate between moderate and severe OED. VEGF expression increased as the disease progressed from normal to increasing grades of OED to malignancy. In OSMF, expression was similar to that in mild OED. VEGF, E-cadherin expression for basal and parabasilar cells showed a strong statistically significant negative correlation in NOM. A very strong statistically significant positive correlation with perfect monotonic relation was noted in superficial cells in severe OED group and OSCC group.

Conclusion: E-Cadherin and VEGF could be used as combination markers to predict the potential risk for malignant transformation in OEDs.

Keywords: E-Cadherin, Oral Epithelial Dysplasia, Oral Squamous Cell Carcinoma and Oral Potentially Malignant Disorders, Oral Submucous Fibrosis, Vascular Endothelial Growth Factor

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide. It constitutes over 90% of malignancies found in the oral cavity and oropharynx.[1] It is the 12th most common cancer in women and sixth most common cancer in men.[2] OSCC is preceded by Oral potentially malignant disorders (OPMDs). The incidence of OPMDs has been reported to be high in the Indian Subcontinent ranging between 0.6/1000 to 30.2/1000. Sir James Paget first reported the malignant transformation of an oral lesion into tongue carcinoma in 1870.[3] The most common PMDs with malignant potential are erythroplakia, oral leukoplakia, oral lichen planus and oral submucous fibrosis.[4]

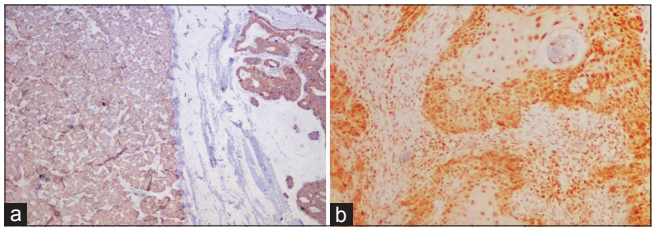

Figure 1: Immuno histochemistry expression of control (a) Breast carcinoma (E-cadherin), (b) oral squamous cell carcinoma (vascular endothelial growth factor)

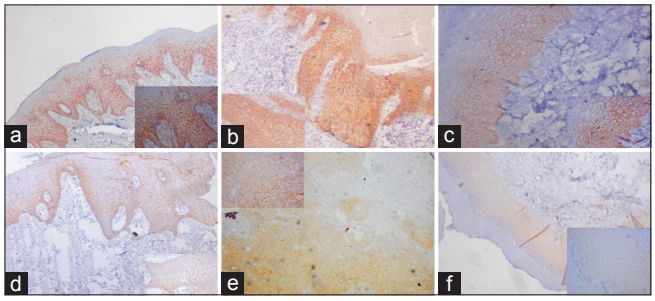

Figure 2: Immuno histochemistry expression of E-cadherin. (a) NOM (×4) (Inset, ×20), (b) mild oral epithelial dysplasia (×4) (Inset, ×20), (c) moderate oral epithelial dysplasia (×4) (Inset, ×20), (d) severe oral epithelial dysplasia (×4) (Inset, ×20), (e) oral squamous cell carcinoma (×4) (Inset, ×20), (f) oral submucous fibrosis (×4) (Inset, ×20)

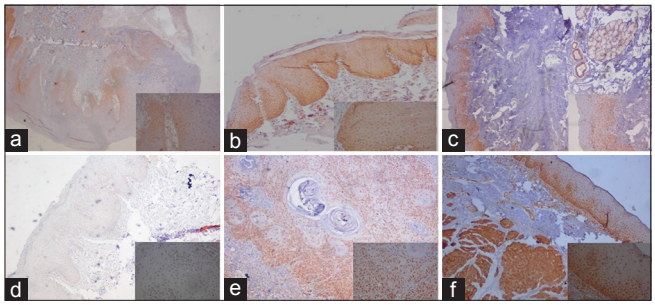

Figure 3: Immuno histochemistry expression of vascular endothelial growth factor. (a) NOM (×4) (Inset, ×20), (b) Mild oral epithelial dysplasia (×4) (Inset, ×20), (c) moderate oral epithelial dysplasia (×4) (Inset, ×20), (d) severe oral epithelial dysplasia (×4) (Inset, ×20), (e) oral squamous cell carcinoma (×4) (Inset, ×20), (f) oral submucous fibrosis (×4) (Inset, ×20)

Oral epithelial dysplasia (OED) has been considered as the progenitor for malignant changes. WHO (2005) has graded OED into mild, moderate and severe based on the architectural changes and cellular atypia at different levels of epithelium.[5] However, there has been a lack of definitive criteria which can be adopted as a gold standard.[6] Malignant transformation in many PMDs is due to loss of epithelial phenotype and decreased differentiation. During this process, epithelial cells acquire a mesenchymal phenotype known as epithelial-mesenchymal transition.[5] Many genes or proteins have emerged over the years as potential markers of dysplasia and/or malignant transformation.[7] Hence, identification of these markers may be a useful tool for prediction of malignant transformation.[8] Two such markers studied over the years with conflicting results are E-cadherin and vascular endothelial growth factor (VEGF).

The cadherins are a family of homophilic cell adhesion proteins expressed in a variety of tissues which require Ca+2 binding for adhesiveness, rigidity and stability.[5] Epithelial cadherin also termed as E cadherin or cadherin 1 is a transmembrane glycoprotein, functioning as a cell adhesion molecule.[9] It is present on the lateral surfaces of epithelial cells in the region of cell–cell contact known as adherens junction.[5] Dysfunctional E-cadherin is associated with loss of differentiation and acquisition of invasive phenotype.[5] Some authors have reported that E-cadherin is potential marker for dysplasia, but some question its reliability.

Angiogenesis is an important phenomenon in OED for nutrition and growth of dysplastic cells. It is initiated by an increase in angiogenic stimulants such as VEGF.[10] VEGF is also known as vascular permeability factor. It belongs to the platelet-derived growth factor (PDGF) family. VEGF stimulates the proliferation of endothelial cells and is important in neovascularization leading to tumor growth and metastasis.[11] Some studies have reported overexpression of VEGF from normal mucosa to different grades of OED to OSCC.[10] However, some other studies report conflicting results, and thus VEGF as a predictor of malignant transformation in OED remains obscure.

Further, the expression pattern of VEGF and E-cadherin also varies considerably in different layers of epithelium. At present, to the best of our knowledge, there exists no literature that compare VEGF and E-Cadherin expression in various grades of OED, oral submucous fibrosis (OSMF), OSCC and normal oral mucosa in different layers of the epithelial thickness. Therefore, the purpose of this

study was to evaluate the Immunohistochemical (IHC) expression of VEGF and E-cadherin at various levels of the epithelium in predicting malignant potential of different grades of OED and OSMF.

MATERIALS AND METHODS

An *in vitro* case–control study was performed on 60 specimens obtained from the archives of the Department of Oral and Maxillofacial Pathology, AECS Maaruti College of Dental Sciences and Research Centre, Bengaluru. The study group was divided into six groups with 10 specimens in each on the basis of clinicopathology as Group I ($n = 10$) – mild ED, Group II ($n = 10$) – moderate ED, Group III ($n = 10$) – severe ED, Group IV ($n = 10$) – oral submucous fibrosis, Group V ($n = 10$) – normal oral mucosa and Group VI ($n = 10$) – OSCC. All the archival specimens were sectioned and stained with hematoxylin and eosin stain to reconfirm the diagnosis. All the slides in Groups I, II and III were graded using the WHO criteria (2005) to reconfirm the grade of dysplasia.

Immunohistochemical technique

The immunohistochemical technique for staining of E-cadherin and VEGF was performed according to the manufacturer's protocol (Pathnsitu). About 3 μm thick sections from tissue blocks of samples and control (breast carcinoma for E-cadherin and OSCC for VEGF) were obtained and mounted on APES coated slides. These slides were incubated at 33°C overnight on the previous day of staining and at 60°C for 1 h on the day of staining. The slides were deparaffanized using three changes of xylene each of 5 min duration and were then hydrated through decreasing grades of isopropyl alcohol (100%, 90% and 70%) and then in distilled water. The tissues were then incubated with peroxide block for 20 min at room temperature to block endogenous peroxide activity and washed in distilled water and Tris buffer for 5 min. The slides were then subjected to antigen retrieval using Tris EDTA buffer (Pathnsitu, Lot. no. A03009MA) supplied along with the kit. The antigen retrieval was carried out in a pressure cooker at 150°C for 55 min. After the retrieval, slides were allowed to cool down to the room temperature. The slide sections were subjected to two washes of Tris buffer for 10 min each and were subsequently incubated for 15 min with protein block to eliminate background staining. The sections required for E-Cadherin were then incubated with E-cadherin primary monoclonal rabbit antibody (Pathnsitu Lot. no. AM3900515) and sections required for VEGF were incubated with VEGF primary monoclonal rabbit antibody (Pathnsitu Lot. no. AR4A31214B) for 30 min and washed with Tris buffer twice for 5 min each. Subsequently, the slides were incubated with Pathnsitu polymer (I04015RB1) for 30 min. The slides were then washed as before and incubated with fresh 3,3'-diaminobenzidine (DAB) chromogen for 2 min. The DAB chromogen was prepared by adding DAB to the buffer at the ratio of 1:20. The slides were then washed in water to stop the chromogen reaction and excess DAB and counterstained with Mayer's Haematoxylin for 6 min. The slides were then dehydrated through graded isopropyl alcohol (70%, 90% and 100%) cleared using xylene and mounted with DPX. The stained sections were viewed under binocular Olympus Research Microscope (BX 41).

Defining positivity of immunohistochemical stain

The presence of brown colored end product (DAB positivity) was indicative of positive immunoreactivity. Cells showing membranous staining were considered positive for E cadherin and cells showing cytoplasmic staining were considered as positive for VEGF. The intensity of expression of positive control was used as a reference to grade the intensity of IHC expression [Figure 1].

Interpretation immunohistochemical expression

The intensity of IHC expression was assessed using the technique described by Allred *et al*.[12] and scores were interpreted as follows: 0 – no positive cells, 1+ – mild intensity, 2+ – moderate intensity and 3+ – strong intensity. The percentage of cells with specific intensity were recorded and multiplied with that particular intensity. The obtained score was tabulated as quick score (QS). The QS was calculated for basal cells, parabasilar cells, superficial cells (including intermediate cells) and corneal cells in all the six groups and also for epithelial cells in the connective tissue stroma of OSCC.

QS = Intensity (I) × Percentage of cells positive in a particular intensity

Statistical analysis

Mean QS for each intensity in all the layers of epithelium for all the groups was calculated and grand mean of QS was then established. The data were used to statistically compare and correlate within and between the groups using Kruskal–Wallis ANOVA and pair-wise comparison with Mann–Whitney U Test. This nonparametric analysis was used as the samples were not normally distributed. Another nonparametric analysis Spearman rank correlation was used to assess the strength of relation between VEGF expression and E-cadherin expression for various grades of OED, OSMF and OSCC.

RESULTS

E-Cadherin demonstrated a heterogeneous pattern of expression. E-cadherin exhibited membranous staining in

basilar cells, parabasilar cells, superficial cells (intermediate cells) and corneal cells [Figure 2]. The grand mean of E-cadherin expression for basal cells showed increased expression in NOM (175 ± 68.72 [mean ± standard deviation (SD)]) with a gradual reduction in expression as the disease progressed from mild ED (149 ± 27.26 [mean ± SD]), moderate ED (99 ± 78.98 [mean ± SD]) to severe ED (58.50 ± 51.75 [mean ± SD]). The grand mean of E-cadherin expression in severe ED and OSCC (component within the epithelium) was almost similar. The grand mean of E-cadherin expression in OSMF was more than that of severe ED and OSCC but less than that of moderate ED. Comparison of grand mean QS of basal cells between six groups using Kruskal–Wallis ANOVA demonstrated a statistical significant difference as $P = 0.0001$. The grand mean of E-Cadherin expression exhibited almost similar pattern of expression as that of basal cells for para basal cells, superficial cells and corneal cells with a P value of 0.0001, 0.001 and 0.0060, respectively as indicated in Table 1. In addition to this, pair-wise comparison made to assess E-cadherin expression between groups using Mann–Whitney *U*-test showed a statistical significance between few groups. The groups which demonstrated a statistical significance for different intensities of expression is described in Table 2.

Further, the epithelial cells in connective tissue stroma of OSCC showed E-cadherin expression to be less than 10% with 1+ intensity in 30% samples of OSCC and 2+ intensity in 40% samples of OSCC. These findings of E-cadherin expression in mild ED, moderate ED, severe ED, OSMF, NOM and OSCC indicate a gradual reduction of E-cadherin expression as disease progressed from NOM to OSCC. These findings were very much evident in basal and para basal cells. E-cadherin expression in corneal cells of NOM and not in any other group indicated loss of E-cadherin expression as disease progressed. However, such E-cadherin expression in corneal cells and superficial cells was less compared to that seen in basal and para basal cells.

VEGF expression also demonstrated a heterogeneous pattern of expression. VEGF showed a predominantly cytoplasmic staining in basilar cells and parabasilar cells of all the six groups [Figure 3]. The superficial cells (Intermediate cells included) showed VEGF expression in all grades of OED and OSCC, whereas corneal cells showed VEGF expression in moderate ED, severe ED and OSCC. The grand mean of VEGF expression for basal cells showed increased expression in severe ED (150.00 ± 0.00 [mean ± SD]) followed by moderate ED (100 ± 00.00 [mean ± SD]) and mild ED (50 ± 00.00 [mean ± SD]). The grand mean of

Table 1A: Comparison of mean QS and grand mean QS of E Cadherin expression between groups using Kruskal-Wallis ANOVA. Basilar cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	5.50	15.71	121.00	61.18	22.50	45.41	149.00	27.26
GROUP 2	3.00	4.22	90.00	77.46	6.00	7.75	99.00	78.98
GROUP 3	2.00	2.58	55.00	49.72	1.50	4.74	58.50	51.75
GROUP 4	47.50	24.86	18.00	46.62	0.00	0.00	65.50	34.19
GROUP 5	5.00	15.81	20.00	42.16	150.00	106.07	175.00	68.72
GROUP 6	2.00	2.58	55.00	49.72	1.50	4.74	58.50	51.75
H	23.6880		17.9630		23.1950		22.8630	
P	0.0001*		0.0030*		0.0001*		0.0001*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 1B: Parabasilar cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	5.50	15.71	121.00	61.18	22.50	45.41	149.00	27.26
GROUP 2	1.50	3.37	90.00	77.46	6.00	7.75	97.50	80.94
GROUP 3	0.50	1.58	55.00	49.72	1.50	4.74	57.00	52.45
GROUP 4	47.50	24.86	18.00	46.62	0.00	0.00	65.50	34.19
GROUP 5	5.00	15.81	25.00	42.49	150.00	106.07	180.00	74.35
GROUP 6	0.50	1.58	55.00	49.72	1.50	4.74	57.00	52.45
H	30.1670		16.6580		23.1950		22.9900	
P	0.0001*		0.00501*		0.0001*		0.0001*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 1C: Superficial cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	5.50	15.71	120.00	63.25	19.50	46.40	145.00	34.32
GROUP 2	0.00	0.00	90.00	77.46	0.00	0.00	90.00	77.46
GROUP 3	0.00	0.00	15.00	47.43	1.50	4.74	16.50	52.18
GROUP 4	47.50	24.86	15.00	47.43	0.00	0.00	62.50	35.84
GROUP 5	5.00	15.81	35.00	47.43	135.00	116.19	175.00	78.17
GROUP 6	0.00	0.00	15.00	47.43	1.50	4.74	16.50	52.18
H	59.0000		39.1650		21.7980		20.8150	
P	0.0001*		0.0001*		0.0010*		0.0010*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 1D: Corneal cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	0.00	0.00	27.00	44.23	0.00	0.00	27.00	44.23
GROUP 2	0.00	0.00	2.00	6.32	0.00	0.00	2.00	6.32
GROUP 3	0.00	0.00	15.00	47.43	0.00	0.00	15.00	47.43
GROUP 4	4.00	5.16	2.00	6.32	0.00	0.00	6.00	6.99
GROUP 5	0.00	0.00	10.00	31.62	34.50	68.57	44.50	70.26
GROUP 6	0.00	0.00	15.00	47.43	0.00	0.00	15.00	47.43
H	21.0710		16.4810		26.7860		16.1940	
P	0.0010*		0.0060*		0.0001*		0.0060*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 2: Groups that showed significant statistical difference between them for E Cadherin expression when compared using Mann-Whitney '*U*' test

1+	2+	3+
Basilar cells		
1 v/s 4	1 v/s 3	1 v/s 4
2 v/s 4	1 v/s 4	2 v/s 5
3 v/s 4	1 v/s 5	4 v/s 5
4 v/s 5	1 v/s 6	
4 v/s 6	2 v/s 5	
Parabasilar cells		
1 v/s 4	1 v/s 3	1 v/s 4
2 v/s 4	1 v/s 4	2 v/s 5
3 v/s 4	1 v/s 5	3 v/s 5
4 v/s 5	1 v/s 6	4 v/s 5
4 v/s 6		5 v/s 6
Superficial cells		
1 v/s 4	1 v/s 3	2 v/s 5
2 v/s 4	1 v/s 4	3 v/s 5
3 v/s 4	1 v/s 5	4 v/s 5
4 v/s 5	1 v/s 6	5 v/s 6
4 v/s 6		
Corneal cells		
Nil	Nil	Nil

1 v/s 4 to be read as Group 1 versus Group 4 and accordingly for all values. Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 3A: Comparison of mean QS and grand mean QS of VEGF expression between groups using Kruskal-Wallis ANOVA. Basilar cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	50.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00
GROUP 2	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00
GROUP 3	0.00	0.00	150.00	0.00	0.00	0.00	150.00	0.00
GROUP 4	20.00	25.82	33.33	66.14	0.00	0.00	50.00	57.74
GROUP 5	2.00	2.58	0.00	0.00	0.00	0.00	2.00	2.58
GROUP 6	0.00	0.00	150.00	0.00	0.00	0.00	150.00	0.00
H	40.0300		50.5960		0.0000		49.4570	
P	0.0001*		0.0001*		1.0000		0.0001*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 3B: Parabasilar cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	50.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00
GROUP 2	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00
GROUP 3	0.00	0.00	150.00	0.00	0.00	0.00	150.00	0.00
GROUP 4	10.00	21.08	15.00	47.43	0.00	0.00	25.00	48.59
GROUP 5	2.00	2.58	0.00	0.00	0.00	0.00	2.00	2.58
GROUP 6	0.00	0.00	150.00	0.00	0.00	0.00	150.00	0.00
H	42.4240		54.6960		0.0000		52.0190	
P	0.0001*		0.0001*		1.0000		0.0001*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

VEGF expression in OSMF and OSCC (intraepithelial component) was similar to that of mild ED and severe ED,

Table 3C: Superficial cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	3.00	4.83	14.00	9.66	0.00	0.00	17.00	4.83
GROUP 2	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00
GROUP 3	0.00	0.00	150.00	0.00	0.00	0.00	150.00	0.00
GROUP 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GROUP 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GROUP 6	0.00	0.00	150.00	0.00	0.00	0.00	150.00	0.00
H	32.7460		15.5260		57.2800		0.0000	
P	0.0001*		0.0080*		0.0001*		1.0000	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

Table 3D: Corneal cells

Groups	1+ (Q.S)		2+ (Q.S)		3+ (Q.S)		Grand mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GROUP 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GROUP 2	40.00	51.64	65.00	47.43	0.00	0.00	105.00	36.89
GROUP 3	0.00	0.00	90.00	77.46	90.00	116.19	180.00	38.73
GROUP 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GROUP 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GROUP 6	0.00	0.00	90.00	77.46	90.00	116.19	180.00	38.73
H	21.0710		26.7700		18.1540		55.7350	
P	0.0010*		0.00011*		0.0030*		0.0001*	

Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

respectively. Basal cells of NOM (2 ± 2.58 [mean ± SD]) showed least expression. Thus, VEGF expression in basal cells showed an increase of expression as the disease progressed from normal to increasing grades of OED to malignancy. Similar expression pattern was also noted in parabasilar cells, superficial cells and corneal cells. Although VEGF expression showed similar expression pattern in superficial cells and corneal cells, there was complete absence of VEGF expression in superficial cells and corneal cells of OSMF group and NOM group as indicated in Table 3. Pair-wise comparison made to assess the VEGF expression between groups using Mann–Whitney U test showed a statistical significance between few groups. The groups which demonstrated a statistical significance for various intensity of expression is described in Table 4. The epithelial cells in connective tissue stroma of OSCC showed heterogeneous expression pattern of VEGF with 100% of cells with 2+ intensity in 70% of samples and 100% of cells with 3+ intensity in 30% of samples of OSCC group. These findings suggest that VEGF expression increased as disease progressed from NOM to increasing grades of OED to OSCC.

The spearman co-relation performed between VEGF expression and E-cadherin expression for basal and para basal cells showed a strong (rho = −0.684) statistically significant negative correlation between VEGF expression

Table 4: Groups that showed significant statistical difference between them for VEGF expression when compared using Mann-Whitney 'U' test

1+	2+	3+
Basilar cells		
1 v/s 2	1 v/s 2	Nil
1 v/s 3	1 v/s 3	
1 v/s 4	1 v/s 6	
1 v/s 5	2 v/s 3	
1 v/s 6	2 v/s 4	
	2 v/s 5	
	2 v/s 6	
	3 v/s 4	
	3 v/s 5	
	4 v/s 6	
	5 v/s 6	
Parabasilar cells		
1 v/s 2	1 v/s 2	Nil
1 v/s 3	1 v/s 3	
1 v/s 4	1 v/s 6	
1 v/s 5	2 v/s 3	
1 v/s 6	2 v/s 4	
	2 v/s 5	
	2 v/s 6	
	3 v/s 4	
	3 v/s 5	
	4 v/s 6	
	5 v/s 6	
Superficial cells		
NIL	1 v/s 2	Nil
	1 v/s 3	
	1 v/s 4	
	1 v/s 5	
	1 v/s 6	
	2 v/s 3	
	2 v/s 4	
	2 v/s 5	
	2 v/s 6	
	3 v/s 4	
	3 v/s 5	
	4 v/s 6	
	5 v/s 6	
Corneal cells		
Nil	Nil	Nil

1 v/s 4 to be read as Group 1 versus Group 4 and accordingly for all values. Group 1- Mild Dysplasia, Group 2- Moderate Dysplasia, Group 3- Severe Dysplasia, Group 4- Oral Sub Mucous Fibrosis, Group 5- Normal Mucosa, Group 6- Oral Squamous Cell Carcinoma

and E-cadherin expression in NOM with $P = 0.0294$ and 0.0289, respectively. A very strong statistically significant positive correlation was noted in superficial cells with a $P = 0.001$ and rho value of 1.000 in severe ED and OSCC indicating a perfect monotonic relation.

DISCUSSION

A normal or dysplastic epithelium acquires tumerogenic or invasive properties due to epithelial mesenchymal transition wherein the epithelial cells acquire a mesenchymal phenotype.[13] Many cell proteins contributing to epithelial mesenchymal transition have been identified over the years through immunohistochemical studies, and one such marker is E- Cadherin.[13,14] This study was conducted to observe and evaluate the expression of E-Cadherin and compare its expression with VEGF expression in six study groups (NOM, Mild epithelial dysplasia, Moderate epithelial dysplasia, Severe epithelial dysplasia, OSMF and OSCC).

In our study all the cases of Normal Oral Mucosa group showed continuous membranous staining in all the cells of basal, parabasal, superficial and corneal cells. E-Cadherin expression reduced from basal to corneal cells. However, expression of E-Cadherin in corneal cells in Normal oral Mucosa suggests for the functional role of E- cadherin in maintaining epithelial tissue integrity5 and loss of its expression indicates a normal desquamation. These observations in our study are consistent with the finding as that of Yogesh *et al* (2011),[15] Yuwanti *et al* (2011),[16] Ahmad *et al* (2013),[7] Zeidler *et al* (2014),[5] Sridevi *et al* (2015)[17] Abdalla *et al* (2017)[18] Da silva *et al* (2017)[8] and Fernandez *et al* (2017).[13]

The grand mean of E-Cadherin expression within layers of epithelium demonstrated similar reduction in the expression of E-Cadherin from basal to corneal cells like in that of NOM in all the groups. The grand mean of E-Cadherin expression compared between various groups indicate a significant reduction expression pattern sequence as disease progressed from normal to OED to malignancy. However, such expression pattern was highlighted with respect to basal and parabasilar layer. These findings of the study was in accordance with the observations in studies by Yogesh *et al* (2011),[15] Yuwanti *et al* (2011),[16] Zeidler *et al* (2014), Abdalla *et al* (2017)[18] Da silva *et al* (2017)[8] and Fernandez *et al* (2017).[13] This decrease in E-Cadherin expression with increase in the severity of dysplasia may be a result of progression of dysplasia and a late event changing towards a cell phenotype with ability to invade (Yogesh *et al*).[15] The behaviour of OSMF as assessed with E-Cadherin expression showed a behaviour intermediate between moderate to severe epithelial dysplasia which was in accordance with the study conducted by Sridevi *et al* (2005).[17]

A significant finding in our study was the E-Cadherin expression of epithelial cells in the connective tissue stroma of OSCC showed less than 10% expression with 1+ intensity in 30% of cases of OSCC and 2+ intensity in 40% of cases of OSCC which was in favour of the study conducted by Yogesh *et al* (2011),[15] Yuwanti *et al* (2011),[16] Ahmad *et al* (2013),[7] Zeidler *et al* (2014)[5] and Sridevi *et al* (2015)[17] and Fernandez *et al* (2017).[13] Yuwanti *et al* [16] observed decreased expression of E-Cadherin in the mucosa adjacent to tumours with respect to normal

mucosa in basal and suprabasal layers. Yogesh *et al* [15] observed negative to patchy staining in small islands and single infiltrating epithelial cells. Ahmad *et al* [7] observed concentric staining within the keratin pearls indicating the gradual loss of E-Cadherin expression from epithelial membranes. Ahmad *et al* [7] and Zeidler *et al* [5] reported a shift of expression from membranous to cytoplasm with advancing histologic grade. The aforesaid findings may be resultant of down regulation of E-Cadherin expression at the transcriptional level leading to transcriptional inactivation and thereby E-Cadherin gene locus being epigenetically silenced by hypermethylation leading to downregulation of E-Cadherin.

The heterogenous pattern of expression was also noticed in VEGF as a predominant cytoplasmic staining in basal and parabasilar cells of Normal Oral Mucosa. The study conducted by Cheng *et al* have shown relatively no expression to minimal positivity of (<2%) of VEGF expression in basal and parabasilar cells in Normal Oral Mucosa. The presences of VEGF expression in the basal and parabasilar component of Normal Oral Mucosa of the present study may be attributed to mild inflammation associated with normal oral mucosa as these were obtained during removal of impacted third molars19. The superficial and corneal cells of Normal mucosa group in this group showed no expression which is in accordance with the studies of Cheng *et al* and sujatha Varma *et al* [19].

There was a steady increase in the expression of VEGF in basal and parabasilar cells as the disease progressed from mild to moderate to severe epithelial dysplasia. In addition to this, the first emergence of VEGF expression in superficial and corneal cells was noted in moderate and severe epithelial dysplasia. These findings are in consistent with the studies of Sujatha Varma *et al* [19] and Sonia Gupta *et al*[10] who also demonstrated VEGF expression in entire thickness of epithelium in severe epithelial dysplasia. This expression of VEGF in all layers of epithelium in severe, moderate and OSCC groups (intraepithelial component) may be attributed o acquisition of transient angiogenic properties required for maintainance of blood supply for development of oral precancerous and cancerous lesions.[20]

OSMF group showed expression of VEGF in basal cells similar to that seen in mild epithelial dysplasia but considerably less to that seen in moderate and severe epithelial dysplasia. The superficial and corneal cells showed no expression similar to normal mucosa which was in support of the findings of Madhavan Nirmal *et al*.[20] This may be due to reduced number of blood vessels in connective tissue stroma in advancing disease process of OSMF[20] as the selected cases of OSMF were of advanced stage. Therefore, it appears that these cells have reduced potential of sustaining angiogenesis.[20] However, staining of basal cells and parabasilar cells with VEGF stain in OSMF group may be due to some grade of epithelial dysplasia present in them. Further, in the present study basal cells in OSCC group(Intraepithelial component) showed the highest expression and the parabasilar cells showed less expression as compared to severe epithelial dysplasia. But the superficial and corneal cells in Severe epithelial dysplasia and OSCC group showed similar expression pattern indicating a morphologically altered epithelium secreting pro-angiogenetic factors much before invasion20and these findings of the study was in favour of the studies conducted by Shivakumar *et al* (2011)[21] Cheng *et al,* (2011)[22] and Torabinia *et al* (2014).[11]

In present study, there was an increased VEGF expression in epithelial cells in connective tissue stroma of OSCC group, which was in par with findings of Penfold and Elisma[23] in HNSCC, and Cheng *et al* (2011)[22]. The present study showed strong intensity of expression in moderate and poorly differentiated OSCC but with less intensity in case of well differentiated OSCC which supports the findings of Sujatha Varma *et al* [19]. This reduced expression in well differentiated SCC may be due to Differentiation of tumor cells to their terminal phenotypes similar to that of keratinocytes.[20] VEGF secreted by tumour cells stimulate tumour growth by increasing the growth and permeability of endothelial cells,[20] the microvessels in tumor environment become leaky and become more permeable for tumor cells. The disintegrated basement membrane and endothelial cells at the tips of growing capillaries Secrete collagenase and plasminogen which in turn increases the likelihood of metastasis.[23]

CONCLUSION

The present study showed increased expression of VEGF and reduced expression of E-Cadherin in basal cells and parabasilar cells with increasing grades of Oral ED to OSCC (intraepithelial component). This may lead to increased acquisition of angiogenic factors which promote growth by neovascularisation and a change in cell phenotype that has potential to invade. These findings, also appear to be early changes in OED according to Yogesh *et al* [15], Sujatha Varma *et al* [19] and Sonia Gupta *et al* [10] Since severe ED and OSCC(intraepithelial component) could be effectively statistically differentiated utilizing grand mean score and mean QS of VEGF and E-Cadherin expression, it is logical to involve both these markers in combination to predict the possibility of malignant transformation in isolated cases of oral severe EDs. However, this has to be

further substantiated with future studies on a larger sample size before adapting such combination of markers routinely to predict malignant transformation in severe OED.

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Conflicts of interest

There are no conflicts of interest.

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